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ATTACHMENT TO CONTINUATION PATENT APPLICATION
FILED UNDER 37 C.F.R. 1.53

ENTITLED: APPARATUS AND METHOD FOR MAKING A PLURALITY OF REAGENT MIXTURES AND ANALYZING PARTICLE DISTRIBUTIONS OF THE REAGENT MIXTURES (As Amended)

INVENTOR: Edward L. Carver, Jr.

DOCKET NO.: 4537-01-2

INCLUDING: Transmittal Sheet

Copy of prior application serial no. 08/370,023 as filed

Transmittal Letter to the Draftsman

4 Sheets Formal Drawings (Figs. 1-6)

Verified Statement Claiming Small Entity (copy from parent application)

Preliminary Amendment

Information Disclosure Statement

Form PTO 1449

Check in the amount of $ 395.00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

A

Docket No: 4537-01-2
Anticipated Classification of this application:
Class: 422 Subclass: 73
Prior Application: 08/370,023
Examiner: A. Soderquist
Art Unit: 1313
BOX PATENT APPLICATION
Hon. Assistant Secretary and Commissioner of Patents and Trademarks
Washington, D. C. 20231

"EXPRESS MAIL" MAILING LABEL NUMBER EM262981096US
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I HEREBY CERTIFY THAT THIS PAPER OR FEE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE "EXPRESS MAIL POST OFFICE TO ADDRESSEE" SERVICE UNDER 37 C.F.R. 1.10 ON THE DATE INDICATED ABOVE AND IS ADDRESSED TO THE COMMISSIONER OF PATENTS AND TRADEMARKS, WASHINGTON, D.C. 20231

Nicole Thorne
(TYPED OR PRINTED NAME OF PERSON MAILING PAPER OR FEE)

Nicole Thorne
(SIGNATURE OF PERSON MAILING PAPER OR FEE

FILING UNDER 37 C.F.R. 1.53

This is a request for filing a
_X_ Continuation
_____ Divisional

application under 37 C.F.R. 1.53, of pending prior application Serial No. 08/370,023 filed on January 9, 1995
of Edward L. Carver, Jr.
for APPARATUS AND METHOD FOR MAKING A PLURALITY OF REAGENT MIXTURES AND ANALYZING PARTICLE DISTRIBUTIONS OF THE REAGENT MIXTURES (As Amended)

1. Copy of Prior Application as Filed Which is Attached

_X_ I hereby verify that the attached papers are a true copy of what is shown in my records to be the above identified prior application, including the oath or declaration originally filed (37 C.F.R. 1.53)

The copy of the papers of prior application as filed which are attached are as follows:
_27_ page(s) of specification
_8_ page(s) of claims
_1_ page(s) of abstract
_4_ sheet(s) of drawing(s)
_2_ page(s) of declaration and power of attorney

If the copy of the declaration being filed does not show applicant's signature indicate thereon that it was signed and complete the following:

_____ in accordance with the indication required by 37 C.F.R. 60(b) my records reflect that the original signed declaration showing applicant's signature was filed on _____

_____ the amendment referred to in the declaration filed to complete the prior application and I hereby state, in accordance with the requirements of 37 C.F.R. 1.60(b), that this amendment did not introduce new matter therein.

2. Amendments

_____ Cancel in this application original claims _____ of the prior application before calculating the filing fee

_X_ A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered original claim in the prior application.)

3. Fee Calculation

	Small Entity	Large Entity
	$395.00	$790.00

Basic Fee
Total Claims: 13 - 20 = 0
(Small $11.00 Large $22.00)

Independent
Claims: 2 - 3 = 0
(Small $41.00 Large $82.00)

Multiple dependent
claims: 0 = 0
(Small $135.00 Large $270.00)

Total $ 395.00 $ _____

_____ Fee for extra claims is not being paid at this time.

4. Small Entity Status
_X_ a verified statement that this filing is by a small entity is:
_____ attached
_X_ filed in the parent application and such status is still proper and desired (37 C.F.R. 1.28(a))

5. Drawings
_____ Transfer the drawings from the prior application to this application and, subject to item 13 below, abandon said prior application as of the filing date accorded this application. A duplicate copy of this request is enclosed for filing in the prior application file. (may only be used if signed by (1) applicant (2) assignee of record or (3) attorney or agent of record authorized by 37 C.F.R. 1.138 and before payment of issue fee).
_____ Transfer the following sheet(s) of drawing from the prior application to this application _____
_X_ New drawings are enclosed.
_X_ formal
_____ informal

6. Priority - 35 U.S.C. 119
_____ Priority of application Serial No. _____ filed on _____ in _____ is claimed under 35 U.S.C. 119.
_____ The certified copy has been filed in prior application Serial No. _____ filed on _____

7. Relate Back - 35/U.S.C. 120
_X_ Amend the specification by inserting before the first line the sentence:
This is a _X_ continuation
_____ divisional
of-pending application Serial No. 08/370,023 filed on January 9, 1995 .

8. Assignment
_X_ The prior application is assigned of record to CDC Technologies, Inc.
Assignment recorded in Patent and Trademark Office on _____
_____ Reel _____ Frame _____
_____ An assignment of the invention to is attached.

9. Fee Payment Being Made At This Time
Not enclosed
_____ No filing fee is submitted. This and the surcharge required by 37 C.F.R. 1.16(e) can be paid subsequently.
Enclosed

_X_	basic filing fee	$ 395.00
_____	recording assignment ($40.00; 37 C.F.R. 1.21 (h)(i))	$ _____
_____	processing and retention fee ($130.00; 37 C.F.R. 1.53(d) and 1.21(1)	$ _____
	Total fees enclosed	$ 395.00

10. Method Of Payment Of Fees
X Enclosed is a check in the amount of $ 395.00
_____ Charge Account No. 13-0235 in the amount of $ _____
A duplicate of this request is attached.

11. Authorization to Charge Additional Fees
X The Commissioner is hereby authorized to charge the following additional fees which may be required to Account No. 13-0235.
X 37 C.F.R. 1.16 (filing fees)
X 37 C.F.R. 1.16 (presentation of extra claims)
X 37 C.F.R. 1.17 (application processing fees)

12. Power of Attorney
X The power of attorney in the prior application is to McCormick, Paulding & Huber LLP
X the power appears in the original papers in the prior application.
_____ since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
_____ a new power has been executed and is attached.
X address all future communications to: Mark D. Giarratana

13. Maintenance of Copendency of Prior Application
_____ A petition, fee and response has been filed to extend the term in the pending prior application until _____
_____ Please abandon the prior application when the petition for extension of time in that application is granted and when this application is granted a filing date so as to make this application copending with said prior application.
_____ Please maintain the prior application so as to make this application copending with said prior application.

14. Conditional Petition for Extension of Time in Parent Application.
_____ A conditional petition for extension of time is being filed in the pending parent application.

Date March 16, 1998

Respectfully submitted,

Mark D. Giarratana

Mark D. Giarratana
Registration No. 32,615
Attorney of Record

McCormick, Paulding & Huber
CityPlace II, 185 Asylum Street
Hartford, Connecticut 06103-4102
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Nicole Thorne
(TYPED OR PRINTED NAME OF PERSON MAILING PAPER OR FEE)

(SIGNATURE OF PERSON MAILING PAPER OR FEE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of)
)
Edward L. Carver, Jr.)
)
on APPARATUS AND METHOD FOR)
MAKING A PLURALITY OF REAGENT)
MIXTURES AND ANALYZING PARTICLE)
DISTRIBUTIONS OF THE REAGENT)
MIXTURES (As Amended))
)
Serial No.: To Be Assigned)
)
Filed on: March 16, 1998) (Our Docket No. 4537-01-2)

Hartford, Connecticut, March 16, 1998

Box: Patent Application
Hon. Assistant Secretary and
Commissioner of Patents and Trademarks
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Dear Sirs:

Prior to examination, please amend the above-identified patent application as follows:

In the title:

Please amend the title to read in its entirety as follows:

-- **APPARATUS AND METHOD FOR MAKING A PLURALITY OF REAGENT**

MIXTURES AND ANALYZING PARTICLE DISTRIBUTIONS OF THE REAGENT MIXTURES --.

In the specification:

On page 1, on the line between the title and the "Field of the Invention", please insert:

-- This patent application is a continuation of U.S. patent application serial number 08/370,023, filed January 9, 1995, co-pending herewith, which is a divisional of U.S. patent application serial no. 08/007,111, filed January 21, 1993, now U.S. Patent No. 5,380,491, both of which are hereby expressly incorporated by reference as part of the present disclosure.--

On page 9, line 9, after "June 7, 1991," please insert -- now U.S. Patent No. 5,316,725, --.

On page 9, line 11, before "co-pending"' please insert -- U.S. Patent No. 5,262,329, entitled "Method For Improved Multiple Species Blood Analysis", which is a continuation of --.

On page 9, line 13, before "which", please insert -- now abandoned --.

On page 9, line 14, please delete "both" and substitute therefor -- each --.

On page 9, lines 25 and 26, please delete "focused flow", and substitute therefor in all capital letters -- FOCUSED FLOW™ --.

On page 14, line 10, please delete "focused flow", and substitute therefor in all capital letters -- FOCUSED FLOW™ --.

On page 15, line 1, after "serial no. 650,686," please insert -- now U.S. Patent No. 5,187,673, --.

On page 23, lines 2 and 3, please delete ", or focused flow of the sample".

On page 23, lines 22 and 23, please delete ", or with systems which do not provide focused flow,".

On page 25, line 10, please delete "focused" and substitute therefor -- unique --.

On page 1, in the "Field of the Invention", please delete from line 5, starting with "The present invention . . . " through line 8, ending with " . . . in hematology testing." and substitute therefor the following:

-- The present invention is directed to apparatus and methods for making reagent mixtures, and more particularly, to apparatus and methods for making a plurality of reagent mixtures and analyzing particle distributions of the reagent mixtures, such as for blood cell analysis. --

On page 3, line 14, please delete "focused flow,".

On page 3, after line 17, please insert the following new paragraph:

-- The present inventors have also realized that it would be desirable to provide an apparatus and method for hematology testing that may automatically adjust or create reagent mixtures corresponding to each of a plurality of different species. --

On page 3, in the "Summary of the Invention", please delete from line 20, starting with "The present invention is directed to . . . " through page 5, line 1, ending with " . . . the pump unit."; and substitute therefor the following:

-- The present invention is directed to an apparatus and method for making a plurality of reagent mixtures and analyzing particle distributions of the reagent mixtures, such as for blood cell analysis. The apparatus comprises at least one pump, such as a positive-displacement pump, a sensing unit defining a counting orifice for receiving a reagent mixture and analyzing a particle distribution of the reagent mixture, and a control unit, or like means, for adjusting the reagent mixture to correspond to each of a plurality of different operator inputs. The control unit controls one or more pumps to aspirate a predetermined quantity of each of a plurality of reagent-mixture components, wherein each predetermined quantity corresponds to the respective input, and further controls the pump or pumps to mix the aspirated components into the reagent mixture. The control unit then controls a pump to introduce the reagent mixture through the sensing unit for sensing a particle distribution of the reagent mixture, such as for blood cell analysis.

In one embodiment of the present invention, the plurality of inputs each correspond to a respective animal species, and for each input, the plurality of reagent-mixture components includes a first reagent-mixture component consisting essentially of a whole blood sample of the respective species, a second reagent-mixture component consisting essentially of diluent, and a third reagent-mixture component consisting essentially of a lysing agent for making a blood/diluent and/or a blood/diluent/lyse reagent mixture corresponding to the respective species. --

On page 5, line 2, please delete "In one" and substitute therefor -- Also in an --, and please delete "processing and"; on line 5, please delete "processing and"; and on line 7, please delete "the pump unit" and substitute therefor -- one or more pumps --, and please delete ", by at least one syringe,".

Also on page 5, please delete from line 10, starting with "One embodiment . . ." through page 7, line 8, ending with "the system.", and substitute therefor the following new paragraph:

-- One advantage of the apparatus and method of the present invention is that the reagent mixture may be automatically adjusted to correspond to each of a plurality of different operator inputs, which may each correspond, for example, to a different animal species to automatically create the reagent mixture for each species. --

On page 10, line 11, after "and includes", please insert -- several pumps, which in the embodiment of the present invention illustrated are positive-displacement pumps, including --; and on line 13, after "piston", please delete the comma ",", before "and", please insert an open parenthesis -- (--, and after "pump", please insert a closed parenthesis --) --.

On page 22, line 14, before "present invention", please insert -- illustrated embodiment of the --.

On page 24, line 17, before "present invention", please insert -- illustrated embodiment of the --; and line 18, after "three", please insert -- pumps or --.

On page 25, line 9, before "present invention", please insert -- illustrated embodiment of the --.

On page 26, line 24, please delete "syringes" and substitute therefor -- pumps --; after "aspirate", please insert a comma "," and delete "and/or"; and after "inject", please insert -- or otherwise pump --.

In the Claims:

Please cancel claims 1-26, and add the following new claims:

27. (New) A method for making a plurality of different reagent mixtures and analyzing particle distributions of the reagent mixtures, wherein each reagent mixture corresponds to a respective operator input, and the method is performed with an apparatus having at least one pump, a sensing unit defining a counting orifice for receiving a reagent mixture and analyzing a particle distribution of the reagent mixture, and a control unit responsive to each operator input to control the at least one pump and sensing unit to make a respective reagent mixture and analyze a particle distribution of the reagent mixture, the method comprising the following steps:

in response to each operator input, selecting one or more of a plurality of lysing agents corresponding to the respective operator input;

pumping with the at least one pump a predetermined volume of the at least one selected lysing agent corresponding to the respective operator input;

pumping with the at least one pump a predetermined volume of at least one other reagent-mixture component corresponding to the respective operator input;

intermixing the predetermined volumes of the at least one lysing agent and the at least one other reagent-mixture component, and in turn creating a reagent mixture corresponding to the respective operator input; and

introducing the reagent mixture through the counting orifice of the sensing unit and sensing a particle distribution of the reagent mixture.

28. (New) A method as defined in claim 27, wherein the reagent-mixture components of a plurality of the different reagent mixtures include (i) blood and (ii) at least one lysing agent, and the method comprises the steps of:

in response to each of a plurality of different operator inputs, selecting the ratio of blood to the at least one lysing agent in the corresponding reagent mixture;

pumping with the at least one pump a predetermined volume of the at least one selected lysing agent corresponding to the respective blood/lysing agent ratio;

pumping with the at least one pump a predetermined volume of blood corresponding to the respective blood/lysing agent ratio; and

intermixing the predetermined volumes of blood and the least one lysing agent, and in turn creating a reagent mixture corresponding to the respective operator input.

29. (New) A method as defined in claim 28, comprising the steps of:

in response to each of a plurality of operator inputs, selecting the ratio of blood to at least one first lysing agent and at least one second lysing agent in the respective reagent mixture;

pumping with the at least one pump a predetermined volume of the at least one first lysing agent corresponding to the respective blood/lysing agent ratio;

pumping with the at least one pump a predetermined volume of the at least one second lysing agent corresponding to the respective blood/lysing agent ratio;

pumping with the at least one pump a predetermined volume of blood corresponding to the respective blood/lysing agent ratio; and

intermixing the predetermined volumes of blood and the first and second lysing agents, and in turn creating a reagent mixture corresponding to the respective operator input.

30. (New) A method as defined in claim 27, further comprising the steps of:

providing a database comprising data indicative of (i) a plurality of animal species, and (ii) a plurality of different reagent mixtures and the predetermined volumes of the reagent-mixture components of each reagent mixture, wherein each reagent

mixture corresponds to one or more of the plurality of animal species;

in response to each operator input corresponding to a respective one of the plurality of animal species, selecting one of the plurality of reagent mixtures corresponding to the respective animal species; and

pumping with the at least one pump the predetermined volumes of the reagent-mixture components, and in turn creating the reagent mixture corresponding to the respective animal species.

31. (New) A method as defined in claim 30, wherein the at least one other reagent-mixture component is blood.

32. (New) A method as defined in claim 30, wherein the at least one other reagent-mixture component includes (i) a predetermined volume of blood, and (ii) a predetermined volume of diluent.

33. (New) A method as defined in claim 30, wherein the reagent-mixture components of the plurality of reagent mixtures are selected from the group including: (i) a blood sample of each of a plurality of different animal species, (ii) diluent, (iii) a first lysing agent, and (iv) a second lysing agent.

34. (New) A method as defined in claim 27, further comprising the steps of:

intermixing the predetermined volumes of the at least one lysing agent and the at least one other reagent-mixture component in a mixing chamber, and in turn creating the reagent mixture in the mixing chamber; and

pumping the reagent mixture from the mixture chamber into the sensing unit for sensing the particle distribution of the reagent mixture.

35. (New) An apparatus for making a plurality of reagent mixtures and analyzing particle distributions of the reagent mixtures, comprising:

at least one pump;

a sensing unit defining a counting orifice for receiving a reagent mixture and analyzing a particle distribution of the reagent mixture; and

means for selecting the ratio of blood to at least one lysing agent for creating a plurality of different reagent mixtures, each corresponding to a different operator input, and for controlling the at least one pump in response to each operator input to pump predetermined volumes of blood and at least one lysing agent in accordance with the blood/lysing agent ratio corresponding to the respective operator input, said means further controlling the at least one pump to

(i) intermix the predetermined volumes of blood and at least one lysing agent and thereby create the reagent mixture corresponding to the respective operator input, and

(ii) introduce the reagent mixture through the counting orifice of the sensing unit for sensing a particle distribution of the reagent mixture.

In the Abstract of the Disclosure:

On page 36, line 15, after "orifice.", please insert -- A control unit is connected to the pump unit for adjusting the reagent mixture to correspond to each of a plurality of different operator inputs, wherein each operator input may correspond to a respective animal species, to automatically create and analyze the reagent mixture for each animal species. --

Remarks

Claims 1-26 have been canceled, and new claims 27-35 have been added. It is respectfully submitted that claims 27-35 are allowable, and an early action to that effect is earnestly solicited.

No additional fee is believed to be required; however, if an additional fee is, or to cover any deficiency in fees paid, authorization is hereby given to charge our deposit account no. 13-0235.

Respectfully submitted,

By _____

Mark D. Giarratana
Registration No. 32,615
Attorney for the Applicant

McCORMICK, PAULDING & HUBER LLP
CityPlace II, 185 Asylum Street
Hartford, CT 06103-4102
(860) 549-5290

Applicant or Patentee: Edw[illegible] Lawrence Carver, Jr. et [illegible] Attorney's Docket No.: CDC-104
Serial or Patent No.:
Filed or Issued: Herewith
For: APPARATUS FOR PUMPING AND DIRECTING FLUIDS FOR HEMATOLOGY TESTING

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9 (f) and 1.27 (c)) — SMALL BUSINESS CONCERN

I hereby declare that I am

[X] the owner of the small business concern identified below:

[ ] an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN CDC Technologies, Inc.
ADDRESS OF CONCERN 1 Great Hill Road
Oxford, Connecticut 06478

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9 (d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled Apparatus For Pumping And Directing Fluids For Hematology Testing by inventor(s) Edward Lawrence Carver, Jr. and David Charles DeCava described in

[X] the specification filed herewith
[ ] application serial no. _____, filed _____.
[ ] patent no. _____, issued _____.

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Edward Lawrence Carver, Jr.
TITLE OF PERSON OTHER THAN OWNER
ADDRESS OF PERSON SIGNING 18 Lisa Drive, Oxford, CT 06478

SIGNATURE Edward Carver Jr. DATE 1-20-93

31,961; Joseph S. Kentoffio, Registration No. 33,189; Joseph A. Fischetti, Registration No. 32,656; F. Tyler Morrison III, Registration No. 36,220; and Mark D. Giarratana, Registration No. 32,615; all of the firm of McCormick, Paulding & Huber, CityPlace II, 185 Asylum Street, Hartford, Connecticut 06103-4102, telephone (203) 549-5290.

Please send all correspondence and direct all telephone calls to:

Mark D. Giarratana
McCORMICK, PAULDING & HUBER
CityPlace II, 185 Asylum Street
Hartford, Connecticut 06103-4102
Phone: (203) 549-5290
Fax: (203) 527-0464

Edward L. Carver, Jr.
President
CDC Technologies, Inc.
1 Great Hill Road
Oxford, CT. 06478

ASSIGNEE CERTIFICATION

In accordance with 37 CFR § 3.73 the Assignee hereby certifies that the evidentiary documents with respect to its ownership have been reviewed and that, to the best of Assignee's knowledge and belief, title is in the Assignee seeking to take this action.

Date: 11 4 93

Edward L. Carver, Jr.
CDC Technologies, Inc.
1 Great Hill Road
Oxford, CT. 06478

APPARATUS FOR PUMPING AND DIRECTING FLUIDS FOR HEMATOLOGY TESTING

Field of the Invention

The present invention relates to apparatus and methods for pumping and controlling the flow of fluids, and more particularly, to apparatus and methods for precisely pumping and controlling the flow of sheath fluids and samples in hematology testing.

Background Information

Prior to the mid-1970s, red blood cell, platelet and white blood cell differential analyses were typically conducted by manual examination, with a technician viewing blood film slides with the aid of a microscope. Since that time, hematological analysis has been automated, making its use both widespread and commonplace.

While the methodologies for automated analysis vary, most often the enumeration and analysis involves subjecting a diluted sample of whole blood to a lysing reagent which stromatolyzes and eliminates the red blood cell population, and simultaneously modifies the cell membranes of the more prevalent white cell subpopulations. This causes differential shrinkage of the different cell types and enables discrimination and sorting thereof. The size and number of white blood cells in the sample are then detected with the aid of an automated analyzer, by pulling the sample fluid through a sensing zone, which is typically adapted to detect the size (volume) and/or opacity of the blood cells in

the sample by electrical or optical differences. The blood cells are counted for a period of time sufficient to gather data for analysis, data points are stored in a memory device, and then analyzed in a processor. The data can then be displayed in the form of a two-dimensional or three-dimensional histogram.

There are various prior art devices for supplying sheath stream and sample fluids to the sensing aperture of a detector. U.S. Patent No. 3,740,143 shows a system employing peristaltic pumping to supply a series of diluted blood samples to a flow cell for white blood cell differentiation and counting. Peristaltic pumping, which operates by the occlusion or squeezing of the pump tubes, does not provide a sufficiently steady-state flow, and can result in damage to the integrity of the cells, further degrading the accuracy of the device.

U.S. Patent No. 4,695,431 also shows an apparatus for supplying fluids to a sheath stream flow cell, which employs a single piston pump to inject the sheath fluid into the flow cell with one side of the pump, and simultaneously aspirate the blood sample through the flow cell with the other side of the pump. The piston pump is driven by a drive cylinder operated by controlling the flow of pressurized fluid. By aspirating the blood sample through the flow cell, the suction forces can distort the cells, thus reducing the accuracy of the device. Also, because the single pump is driven by a pressurized cylinder, the fluid quantity cannot be controlled as accurately as may be desired.

For cell or particle analyses of this type, the present

inventors have realized that it is advantageous to detect one cell at a time, and accumulate data on thousands of cells. Coincidence, or the simultaneous passage of multiple cells through the sensing zone, can create anomalies or aberrant information. Although this type of information can be partially corrected by using mathematical equations or pulse editing circuits when analyzing the data, important information about the cells may be rejected and thrown away with the sample. This may include information about abnormalities in the sample, since the abnormal cells may give rise to unusual pulses that are rejected in compensating for the passage of multiple cells through the sensing zone. The present inventors have realized that it would be desirable to provide a precisely controlled, steady-state flow of both blood sample and sheath fluids, focused flow, wherein the sample cells are injected through the sensing zone in a substantially single-file relationship relative to each other in order to avoid coincidence and permit accurate detection of cell properties.

Summary of the Invention

The present invention is directed to an apparatus for hematology testing, comprising a sensing unit, which includes a counting orifice for the flow of a blood sample through the counting orifice in order to analyze the blood sample. A pump unit of the apparatus includes at least two syringes, a first syringe coupled in fluid communication with the sensing unit on the inlet side of the counting orifice for injecting a stream of blood sample

through the counting orifice, and a second syringe also coupled in fluid communication with the sensing unit on the inlet side of the counting orifice for simultaneously injecting a sheath of fluid surrounding the sample stream on the inlet side of the counting orifice.

In one embodiment of the present invention, the pump unit further includes a third syringe coupled in fluid communication with the sensing unit on the outlet side of the counting orifice for aspirating the fluids injected through the counting orifice from the outlet side of the counting orifice. The pump unit preferably includes a single drive motor coupled to both the first, second, and third syringes for simultaneously actuating the syringes. In one embodiment of the present invention, the drive motor is coupled to a threaded shaft and the threaded shaft is coupled to the first, second, and third syringes. Rotation of the drive motor causes rotation of the threaded shaft and simultaneous actuation of the syringes.

In one embodiment of the present invention, the apparatus further comprises a valve matrix coupled between the pump unit and the sensing unit for controlling the flow of fluids between the pump unit and the sensing unit. The apparatus also preferably further comprises a processing and control unit coupled to the pump unit and the sensing unit for controlling actuation of the syringes. One embodiment of the present invention also comprises a first lysing agent container and a second lysing agent container, each being coupled to the pump unit for aspirating the lysing

agents by the syringes of the pump unit.

In one embodiment of the present invention, the processing and control unit includes a database pertaining to predetermined quantities of lysing agents necessary for formulating blood/reagent mixtures for a plurality of species. The processing and control unit is responsive to an input indicating a specific species to control the pump unit to aspirate, by at least one syringe, predetermined quantities of the lysing agents from the first and second lysing containers corresponding to the respective species.

One embodiment of the present invention further comprises a sample probe coupled to at least one syringe of the pump unit for aspirating a predetermined volume of blood sample into the probe for testing. A mixing chamber is also preferably coupled in fluid communication with the pump unit for receiving the lysing agents injected by at least one syringe of the pump unit for mixing the lysing agents with a blood sample.

In one embodiment of the present invention, the sensing unit includes a first injector tube coupled in fluid communication with the first syringe and located on the inlet side of the counting orifice for injecting the sample stream through the counting orifice. A second injector tube is coupled in fluid communication with the second syringe and located on the inlet side of the counting orifice for injecting the sheath fluid adjacent the sample stream. In one embodiment of the present invention, the sensing unit defines an inlet chamber coupled in fluid communication with the counting orifice, the first injector tube, and the second

injector tube. Preferably, in this embodiment, at least a portion of the second injector tube is oriented substantially on a tangent to a surface defining the inlet chamber for injecting the sheath of fluid in a generally spiral path surrounding the sample stream.

In one embodiment of the present invention, the sensing unit also includes a sheath tube coupled in fluid communication with the third syringe and located on the outlet side of the counting orifice for aspirating a sheath of fluid through the sheath tube surrounding the sample stream injected on the outlet side of the counting orifice. In this embodiment, the sensing unit may further include an exit chamber coupled in fluid communication with the outlet side of the counting orifice for receiving the sample/sheath mixture injected through the counting orifice. Preferably, the sheath tube is coupled in fluid communication with the exit chamber, and is oriented substantially on a tangent to a surface defining the exit chamber for directing the sheath of fluid in a generally spiral path surrounding the sample stream on the outlet side of the counting orifice.

One advantage of the present invention, is that the plurality of syringes provides a substantially steady-state flow, which, in combination with the sheath of fluid, provides a fine, narrow sample stream through the center of the counting orifice, significantly enhancing the ability of the sensing chamber (or focused flow cell) to accurately analyze the sample cells. The smooth and precise operation of the syringes, preferably in combination with a single drive motor, provides smooth and precise

control over the flow of fluids through the sensing chamber.

Another advantage of the present invention, is that because the sample and sheath are injected through the inlet side of the counting orifice (as opposed to either being pulled or aspirated only through the orifice), deformation of the cells as they flow through the orifice is substantially avoided. Cell distortion is therefore reduced to a minimum, further enhancing the accuracy of the system.

Other advantages of the present invention will become apparent in view of the following detailed description and accompanying drawings.

Brief Description of the Drawings

Figure 1 is a schematic diagram of an apparatus embodying the present invention.

Figure 2 is front plan view of the pump unit of the apparatus of Figure 1.

Figure 3 is a side plan view of the pump unit of Figure 2.

Figure 4 is a magnified, partial schematic view of the sensing chamber of the apparatus of Figure 1 illustrating schematically the flow of the sheath fluids and of the sample stream.

Figure 5 is a partial detailed cross-sectional view of the sensing chamber of Figure 1.

Figure 6 is a cross-sectional view of Figure 5 taken along the line 6-6 of Figure 5.

Detailed Description

In Figure 1, an apparatus embodying the present invention is indicated generally by the reference numeral 10. The apparatus 10 is employed for hematological testing, and is specifically suited for cell analysis on a wide variety of species. The apparatus 10 includes a sample probe 12 for aspirating a sample of blood to be tested. The sample probe 12 is coupled to a valve matrix 14, which in turn couples the sample probe to a selected syringe within a pump unit 16 to aspirate a predetermined volume of the blood sample into the probe (e.g., 20 μl), as is described further below.

The blood sample is discharged into a mixing cuvette 13, in which a predetermined volume of diluent and a predetermined volume of lytic reagents are rapidly admixed with the whole blood sample. The mixing cuvette 13 is coupled through the valve matrix 14 and the pump unit 16 to a first chamber or container 18 containing a first lysing agent A, and a second chamber or container 19 containing a second lysing agent B. The sample probe 12 is also coupled through the valve matrix 14 and pump unit 16 to a diluent reservoir or container 17. Thus, the sample probe 12 dispenses the blood sample along with a predetermined volume of diluent from the diluent reservoir 17 into the mixing cuvette 13. At about the same time, predetermined volumes of lysing agent A and/or lysing agent B are aspirated from the lysing chambers 18 and 19, respectively, by the pump unit 16, and injected through the valve matrix 14 into the mixing cuvette 13, along with the blood sample and diluent to

formulate the sample blood/reagent mixture, as described further below. Preferably, the ratio of the individual lyse components in the lytic reagent composition are present in a ratio and quantity sufficient to effect at least a component separation of white blood cells, so that they can be differentiated, and at least one of the white blood cell subpopulations can be quantified.

The particular reagent compositions and the preferred methods for employing these compositions are disclosed in co-pending patent application serial no. 711, 965, filed June 7, 1991, entitled "Method And Reagent System For The Improved Determination Of White Blood Cell Subpopulations", and co-pending patent application serial no. 714,671, filed June 13, 1991, entitled "Method And Reagent System For Improved Multiple Species Blood Analysis", which are both hereby expressly incorporated by reference as part of the present disclosure.

The sample blood/reagent mixture remains in the mixing cuvette 13 for a short but sufficient amount of time for the red blood cells to be stromatolyzed and to release their hemoglobin, and for the active lytic reagents to act on the cell membranes of the white blood cells and cause them to selectively separate. After this short time period (e.g., approximately 10 to 30 seconds), the sample is aspirated through the valve matrix 14 into a selected syringe in the pump unit 16, as is described further below. The sample is then injected by the pump unit 16 back through the valve matrix 14 and into a sensing chamber 20 (also referred "focused ~~flow~~" cell) along with a diluent sheath, in which the white blood

cells are counted and the volume (size) and/or opacity is measured by electrical or optical differences. The white blood cells are counted for a period of time to gather sufficient data for analysis, typically about 10,000 cells. Data points are stored and analyzed in a processing and control unit 22, and the data can in turn be visually displayed on a display unit 24. A keyboard unit 25 is coupled to the processing and control unit 22 to control its operation. After the sample is analyzed, it is passed through the valve matrix 14 into a waste container 26 and discarded.

Turning to Figures 2 and 3, the pump unit 16 is illustrated in further detail, and includes a first syringe 28, a second syringe 30, and a third syringe 32. Each of the syringes has a piston, and functions as a positive-displacement pump, which can be coupled through the valve matrix 14 to any of the other fluid-containing components of the apparatus 10 in order to aspirate and/or inject the fluids, as is described further below.

One end of the first syringe 28 is mounted to a base plate 34 by a first base mount 36, and a first piston 38 of the first syringe is mounted on the other end to a drive plate 40 by a first drive mount 42. Similarly, one end of the second syringe 30 is mounted to the base plate 34 by a second base mount 44, and a second piston 46 of the second syringe is coupled on the other end to the drive plate 40 by a second drive mount 48. The third syringe 32, on the other hand, is mounted in the opposite direction of the first and second syringes, 28 and 30, respectively. A piston 50 of the third syringe 32 is coupled on one end to the

drive plate 40 by a third drive mount 52, and the other end of the third syringe is coupled to the base plate 34 by a third base mount 54. By driving the drive plate 40 in the downward direction of Figure 2, the first syringe 28 and second syringe 30 simultaneously inject fluid from the respective syringe, and the third syringe 32 simultaneously aspirates fluid into the syringe, whereas movement of the drive plate 40 in the upward direction of Figure 2 causes the opposite to occur.

The first syringe 28 is coupled to one end of a first line 56 and coupled to one end of a second line 58 by means of a first connector 60. The other end of each of the first line 56 and second line 58 (not shown) is coupled to the valve matrix 14. The second syringe 30 likewise is coupled to one end of a first line 62 and coupled to one end of a second line 64 by means of a second connector 66. The other end of each of the first line 62 and second line 64 (not shown) is coupled to the valve matrix 14. Each of the second lines 58 and 64 are typically used for injecting and/or aspirating fluids with the respective syringe, whereas the first lines 56 and 62 are typically used for purposes of flushing diluent from the diluent reservoir 17 to clean the respective syringe and/or to wash away any air bubbles. The third syringe 32 is coupled to one end of a third line 68 for aspirating and/or injecting fluid with the third syringe. The other end of the third line 68 (not shown) is coupled to the valve matrix 14 for controlling flow through the third line.

As shown in Figure 3, a motor 70 is coupled to the drive plate

40 to precisely move the drive plate, and in turn simultaneously control the actuation of the three syringes. In the embodiment of the present invention illustrated, the motor 70 is preferably an electric stepping motor, but may be a DC or AC electric motor with proper feedback and electronic control, and is coupled to a control board 72, which is in turn coupled to the processing and control unit 22 to control the operation of the motor. The motor 70 comprises a drive shaft 74 coupled to one side of a drive belt 76, which is in turn coupled to one end of a threaded shaft 78 by means of a gear 80. The drive belt 76 preferably defines a plurality of teeth on its inside surface which mesh with corresponding teeth on both the drive shaft 74 and the gear 80 in order to maintain precise control over the movement of these components. The drive shaft 78 is mounted on each end by bearing blocks 82 to the base plate 34, and a drive block 84 is mounted on the threaded shaft 78. The drive block 84 includes an aperture 86 extending through the drive block and defined by a threaded surface 88, which engages the corresponding threads on the threaded shaft 78. The drive plate 40 is coupled to the drive block 84 by drive mounts 90, which extend through an elongated aperture 92 defined within the base plate 34, indicated in dashed lines in Figure 3. As can be seen, the elongated aperture 92 extends in a direction substantially parallel to the threaded shaft 78.

By operating the stepping motor 70, the threaded shaft 78 is rotated to drive the drive block 84 along the axis of the threaded shaft 78 by engagement of the threads on the surface 88 with the

threads on the shaft 78, which in turn simultaneously moves the drive plate 40 and the pistons of the three syringes. Based on the direction of the motor 70, the first and second syringes 28 and 30, respectively, inject, and the third syringe 32 simultaneously aspirates, or vice-versa.

The pump unit 16 further includes a sensor mounted adjacent the threaded shaft 78 and drive block 80 to indicate the position of the drive block and control the operation of the motor 70 in response. The sensor is mounted on a sensor control board 93, which is coupled to the drive control board 72 to transmit signals to the drive control board for controlling the operation of the motor 70. The drive control board 72 is in turn coupled to the processing and control unit 22. The sensor includes three position sensors, a home-position sensor 94, a first-end position sensor 96, and a second-end position sensor 98. The drive block 84 includes a corresponding sensor plate or flag 100 mounted in line with the three position sensors, and adapted to cause each position sensor to generate a signal when the sensor plate is aligned with a respective position sensor.

Accordingly, when the drive block 84 is located in the home position, the home-position sensor 100 transmits a signal indicative of this condition to the control board 72. At this point, the processing and control unit 22 is responsive to this signal to recognize that both the first and second syringes are nearly empty, and the third syringe is nearly full. By counting the number of steps upon operation of the stepping motor 70 from

the home position, the processing and control unit 22 can cause a precise volume of fluid to either be injected or aspirated with each syringe. The first-position sensor 96 and second-position sensor 98 are provided as a safety back-up, each being located at one extreme of the permissible movement of the drive block 84. If either of these position sensors are activated, the drive control board 72 is responsive to stop the motor 70 to prevent any damage to the pump unit 16.

Turning to Figures 4-6, a portion of the sensing chamber or focused flow cell 20 is illustrated in further detail. The sensing chamber 20 includes a sensing zone 102, which defines a counting orifice 104, as shown in Figure 4. The counting orifice 104 receives a narrow stream of the blood sample injected by one of the syringes of the pump unit 16 through the valve matrix 14 and a sample tube 106. The sample tube 106 is substantially coaxial with the counting orifice 104 and injects a narrow stream of the blood sample through the center of the orifice, as illustrated in Figure 4. The sensing zone comprises a transducer (not shown) for detecting differences in electrical, optical, chemical or other characteristics in each of the cells of the sample, and for generating a signal whose characteristics relate to such differences. These signals are transmitted to the processing and control unit 22 where they are processed to determine the parameters of the constituent subpopulations of the sample.

The preferred components of the processing and control unit 22 for performing this function are described in co-pending patent

application serial no. 650,686, filed February 5, 1992, entitled "Method And Apparatus For Determining The Distribution Of Constituent Subpopulations Within A Population Of Particles Having Overlapping Subpopulations", which is hereby expressly incorporated by reference as part of the present disclosure.

As shown in Figures 4 and 5, the sensing chamber 20 includes a sensing unit 101, which defines a substantially conical-shaped surface 108 for receiving a sheath of diluent surrounding the sample stream injected by the sample tube 106. The sensing unit 101 is mounted by means of a pair of o-rings 103 within a support block 105. The support block 105 defines a generally cylindrical chamber A coupled in fluid communication, and substantially concentric with the conical-shaped surface 108. As shown in Figure 5, the sample tube 106 is mounted within the center of the chamber A and extends into the recess defined by the conical-shaped surface 108. A first sheath or diluent tube 110 is coupled on one end to the chamber A, and in the embodiment of the present invention illustrated, is oriented substantially along a tangent to the cylindrical surface defining the chamber A, as shown in Figure 6. The other end of the first sheath tube 110 is coupled through the valve matrix 14 to another of the syringes in the pump unit 16, for receiving a predetermined volume of diluent injected by that syringe. Because the first sheath tube 110 is oriented on a tangent to the cylindrical surface of the chamber A, the diluent follows a generally spiral or helical path through the chamber A, along the conical-shaped surface 108, and through the counting

orifice 104 surrounding the sample stream, as indicated by the arrows in Figure 4. This particular orientation of the first sheath tube 110 is only exemplary, however. For example, one or more first sheath tubes may equally be oriented so that the diluent flows straight along the conical-shaped surface 108 and through the counting orifice 104.

The sensing unit 101 defines a tapered surface 111 on the exit side of the counting orifice 104, which is coupled in fluid communication with a chamber B for receiving the sample stream and diluent injected through the counting orifice. An exit tube 112 is coupled on one end to the chamber B, and is coupled on the other end (not shown) through the valve matrix 14 to a selected syringe of the pump unit 16 to aspirate the sample/diluent mixture injected through the counting orifice 104 into the chamber B and exit tube 112, into the respective syringe.

A second sheath tube 114 is coupled on one end in fluid communication with the chamber B between the o-ring 103 and the exit tube 112. As with the first sheath tube 110, in the embodiment of the present invention illustrated, the second sheath tube 114 is oriented substantially on a tangent to the cylindrical surface defining the chamber B so that the diluent is aspirated into the chamber B and exit tube 112 along a substantially spiral or helical path surrounding the sample stream injected through the counting orifice 104. As with the first sheath tube 110, this particular configuration of the second sheath tube 114 is only exemplary, however. The other end of the sheath tube 114 is

coupled through the valve matrix 14 to the diluent reservoir 17.

As shown in Figure 5, a third diluent tube 115 is coupled to the chamber A between the o-ring 103 and the first diluent tube 110, and is likewise oriented along a tangent to the cylindrical surface defining the chamber A. The other end of the third diluent tube 115 is coupled through the valve matrix 14 to the diluent reservoir (or syringe) for flushing the sensing chamber 20 with fresh diluent after running a sample.

In the operation of the apparatus of the present invention, a whole blood sample is introduced into the sample probe 12. The processing and control unit 22 connects the sample probe 12 to a selected syringe of the pump unit 16 through the valve matrix 14, and then controls the motor 70 to aspirate a predetermined volume of the whole blood sample (e.g., 20 μl) through the probe. The processing and control unit 22 also connects the same syringe through the valve matrix 14 to the diluent reservoir 17, and controls the motor 70 to aspirate a predetermined volume of diluent into the whole blood sample. The processing and control unit 22 then controls the motor 70 to inject the sample/diluent mixture from the syringe through the valve matrix 14 into the mixing cuvette 13.

The processing and control unit 22 has in a database information as to the predetermined volumes of lysing agent A and lysing agent B necessary to form the proper sample blood/reagent mixture for selected animal species (e.g., dog, cat, rat, mouse, horse, cow, rabbit, monkey, pig, goat, bird, etc.). The operator

inputs through the keyboard unit 25 the particular animal species of the blood sample, and the processing and control unit 22 is responsive to this input based on the information in its database to select a predetermined volume of lysing agent A and a predetermined volume of lysing agent B. The processing and control unit 22 then connects a selected syringe in the pump unit 16 to the lysing agent A chamber 18 through the valve matrix 14, and controls the motor 70 (by counting a predetermined number of steps) to aspirate the predetermined volume of lysing agent A by the syringe. The processing and control unit 22 then connects the same syringe (or another syringe) to the lysing agent B chamber 19 through the valve matrix 14, and controls the motor 70 (by counting a predetermined number of steps) to aspirate the predetermined volume of lysing agent B by the syringe. The processing and control unit 22 then couples the syringe through the valve matrix 14 to the mixing cuvette 13, and controls the motor 70 to inject the predetermined mixture of lysing agent A and lysing agent B into the mixing cuvette with the blood/diluent mixture.

After the sample blood/reagent mixture is prepared in the mixing cuvette 13, the processing and control unit 22 couples the second line 58 of the first syringe 28 in fluid communication with the mixing cuvette 13 by actuating a valve (not shown) in the valve matrix 14 to aspirate a predetermined volume of sample blood/reagent mixture into the first syringe. In the embodiment of the present invention illustrated, the volume of the first syringe 28 is 250 μl. The processing and control unit 22 also

couples the second inlet line 62 of the second syringe 30 in fluid communication with the diluent reservoir 17 by actuating a valve (not shown) in the valve matrix 14, to aspirate a predetermined volume of diluent into the second syringe. In the embodiment of the present invention illustrated, the volume of the second syringe 30 is approximately 250 μl. The precise volume of fluid aspirated into each syringe is controlled by the processing and control unit 22, which counts the number of steps of the motor 70 with respect to the home position as indicated by the home-position sensor 94, wherein each step corresponds to a precise volume of fluid for each syringe.

The valves permitting this aspiration of the first and second syringes 28 and 30, respectively, are then closed, and the processing and control unit 22 actuates additional valves in the valve matrix 14 to couple the second line 58 of the first syringe 28 in fluid communication with the sample tube 106 of the sensing chamber 20, and to couple the second line 64 of the second syringe 30 in fluid communication with the first diluent tube 110 of the sensing chamber 20. The processing and control unit 22 also then actuates selected valves of the valve matrix 14 to couple the third line 68 of the third syringe 32 in fluid communication with the exit tube 112 of the sensing chamber 20, and to couple the second diluent tube 114 in fluid communication with the diluent reservoir 17. The system is then ready to analyze the sample.

The processing and control unit 22 then actuates the motor 70 to drive the drive block 84 back toward the home position. This

in turn causes the first and second syringes 28 and 30, respectively, to simultaneously inject the sample blood/reagent mixture through the sample tube 106 and the diluent through the first diluent tube 110. As illustrated in Figure 4, a narrow stream of blood/reagent mixture is thus injected by the first syringe 28 through the center of the counting orifice 104, and a stream of diluent is injected by the second syringe 30 into the chamber A, along the substantially conical-shaped surface 108, and through the counting orifice 104 along a path which surrounds the stream of sample blood/reagent mixture, but substantially avoids any intermixing of the two streams. In the embodiment of the present invention illustrated, the counting orifice is approximately 60 microns in diameter, the sample stream is approximately 15 microns in diameter, and the sheath of diluent is therefore approximately 35 microns thick within the counting orifice, surrounding the sample stream.

Because both the flow of the diluent sheath injected through the first diluent tube 110 is substantially laminar, and the sample stream injected through the sample tube 106 is substantially laminar, there is substantially no intermixing of the two streams. The steady state flow generated by the syringes significantly facilitates in producing a substantially laminar flow. Also, because the sample stream is located in the approximate center of both the recess defined by the conical-shaped surface 108 and the counting orifice 104, it moves at a relatively faster velocity through the counting orifice than does the surrounding sheath of

diluent, thus further preventing any intermixing of the sample and diluent. Also, the sheath of diluent, which in the embodiment of the present invention illustrated follows a generally spiral or helical path as it is injected through the first diluent tube 110, the chamber A, and along the conical-shaped surface 108, surrounds the sample stream with a substantially laminar flow, and thus tends to maintain the sample flow in a fine, substantially uniform stream located in the center of the counting orifice. The flow of the diluent sheath also tends to maintain the sample stream within the center of the counting orifice.

As the first and second syringes 28 and 30, respectively, simultaneously inject the sample and the diluent into the front end of the sensing chamber 20, the third syringe 32 simultaneously aspirates the sample/diluent mixture injected into the outlet side of the counting orifice 104, and also aspirates a second sheath of diluent through the second diluent tube 114 into the exit tube 112. Because the second diluent tube 114 is oriented along a tangent to the surface defining the chamber B, the second sheath of diluent follows a substantially spiral flow path surrounding the sample stream exiting the counting orifice 104. The substantially laminar flow of the second sheath acts to further maintain the sample in a fine, narrow stream as it exits the counting orifice, thus further increasing the ability of the sensing chamber to accurately analyze the sample cells.

The processing and control unit 22 stops the motor 70 after a predetermined volume of the blood sample has been injected by the

first syringe 28. In the embodiment of the present invention illustrated, the volume of the third syringe 32 is approximately 5 ml, which is sufficient to receive the entire volume of fluid injected by both the first and second syringes 28 and 30, respectively, and to aspirate a sufficient volume of diluent through the second diluent tube 114 to form the second diluent sheath in the exit tube 112.

The processing and control unit 22 then actuates a selected valve in the valve matrix 14 to couple the third line 68 of the third syringe 32 in fluid communication with the waste reservoir 26, and the motor 70 is then actuated in the opposite direction (i.e., away from the home position) to expel the sample/diluent mixture in the third syringe into the waste reservoir 26.

One advantage of the present invention, is that because the three syringes are simultaneously driven by the stepping motor, there is a simultaneous, steady-state flow of both the sample and diluent through the counting orifice. The smooth and precise operation of the stepping motor in combination with the direct drive of the threaded shaft and drive plate and the positive-displacement pumping of the syringes, permits precise, simultaneous control of the fluid flow through the sensing chamber. As a result, the flow of both diluent sheaths and the sample stream is substantially laminar, thus substantially preventing any mixing of these fluids within the counting orifice. Also, as described above, the first sheath of diluent can be injected, or aspirated, along a substantially spiral path through the counting orifice and

surrounding the sample stream (which can be aspirated or injected), which also facilitates in maintaining a fine, narrow sample stream, or focused flow of the sample through the counting orifice. Moreover, the second sheath of diluent, which is likewise substantially laminar, and can be injected or aspirated along a substantially spiral path surrounding the sample stream, further contributes to maintaining a fine, narrow sample stream as the sample exits the counting orifice.

Thus, there is a steady, repeatable flow of sample and diluent through the counting orifice each time a sample is injected. Moreover, because the sample stream is maintained in a fine, narrow configuration, the platelets, red blood cells or white blood cells of the sample are oriented in a substantially single file relationship relative to each other as they pass through the counting orifice, thus permitting the sensing chamber to detect approximately one cell at a time, and accumulate data in this fashion on thousands of cells. Accordingly, coincidence, or the passage of multiple cells at once through the counting orifice is substantially avoided. Moreover, the cells flow through the counting orifice in a substantially steady state, which is repeated from one sample to the next. The anomalies or aberrant information normally associated with coincidence, or with systems which do not provide focused flow, are substantially avoided. In addition, because substantially each cell in the sample is detected (due to the substantially single file relationship of the cells), important information is not rejected, enabling the system to provide a more

true measurement of the cell distribution within each sample.

Another advantage of the embodiment of the present invention illustrated, is that because only one drive has to be used to simultaneously drive all three syringes, which then assures that all three syringes move simultaneously. Also, there is a significant cost savings as opposed to a system in which a separate drive may be employed for each syringe.

Yet another advantage of the embodiment of the present invention illustrated, is that because the sample and sheath are injected through the inlet side of the counting orifice (as opposed to either being pulled or aspirated only through the orifice), deformation of the cells as they flow through the orifice is substantially avoided. Cell distortion is therefore reduced to a minimum, further enhancing the accuracy of the system and providing hematocrit measurements that accurately correlate with spun hematocrits.

Yet another advantage of the present invention, is that the three syringes can be employed to inject and/or aspirate different fluids simply by adjusting the connections with the syringes in the valve matrix. For example, it may be desirable to employ the second syringe 30 to inject diluent through the second diluent tube 114. In this case, the first diluent sheath is aspirated through the counting orifice by the third syringe 32, whereas the sample stream and the second sheath are injected by the first and second syringes, respectively. It may equally be desirable to mount the third syringe 30 in the same direction and in the same fashion to

the drive plate 40 and base plate 34 as are the first and second syringes 28 and 30, respectively. In this case, the first syringe 28 may inject the sample, the second syringe 30 may inject the first diluent sheath, and the third syringe 32 may inject the second diluent sheath. In this situation, the exit tube 112 would be coupled in fluid communication with the waste reservoir 26 in order to release the sample/diluent mixture directly into the waste reservoir.

Another advantage of the present invention is that the steady state, focused flow produced by the syringes minimizes protein build-up and clogging. First, the first and second diluent sheaths maintained around the sample cells prevents contact of the sample cells with the walls of both the sensing chamber and the exit tube. Second, because the sample blood/reagent mixture is powerfully injected through the counting orifice by one of the syringes, clogs and build-up within each cycle are prevented.

Another advantage of the present invention, is that the processing and control unit can automatically optimize sample analysis on a species-by-species basis. The database of the processing and control unit can contain information on the predetermined volumes of the lysing agents for all species encountered in this type of hematology system. Thus, the operator does not need to be concerned with preparing the specific blood/reagent mixture for each type of species being tested. Rather, the operator simply inputs the type of species on the keyboard unit, and the processing and control unit automatically

determines the quantities of the lysing agents based on the type of species, and then automatically controls the operation of the pump unit to aspirate the predetermined volumes of lysing agents, and to mix them with the sample/diluent mixture in the mixing cuvette.

In one embodiment of the present invention, the keyboard unit 25 has separate keys for certain species (e.g., cat and dog) and another key for other species. Thus, by pressing the "cat" key or the "dog" key, the processing and control unit automatically causes the preparation of the blood/reagent mixture for the respective species. By pressing the "other" key, the display unit displays the additional species may be processed. Once the correct species is selected, the processing and control unit automatically causes the preparation of the blood/reagent mixture for the respective species.

Another advantage of the present invention is the flexibility of the system to adapt to automatically analyze samples from numerous types of species, and to optimize any cycle for a given species. For example, the lyse volumes, the volume of diluent, and the volume of the whole blood sample, can be easily adjusted simply by controlling the processing and control unit. This can be extremely beneficial for analyzing species that are very different, such as mammalian vs. non-mammalian. Moreover, because the pump unit employs several different syringes to aspirate and/or inject these fluids, the apparatus can automatically mix two or more of these fluids in predetermined volumes, which are precisely measured

by monitoring operation of the stepping motor, which is a significant advantage over prior hematology systems. As illustrated above, the volume of lyse A and/or the volume of lyse B (and other lyse agents may be added if necessary) can be automatically adjusted and mixed with the blood/diluent mixture in the mixing cuvette to effect proper separation of blood cells on a species-by-species basis. This means for variably adjusting the volume of lytic agents is significant in obtaining the proper separation of the white blood cell populations.

Claims:

1. An apparatus for hematology testing, comprising:

a sensing unit defining a counting orifice for the flow of a blood sample through the counting orifice to analyze the blood sample; and

a pump unit including at least two syringes, a first syringe being coupled in fluid communication with the sensing unit on the inlet side of the counting orifice for injecting a stream of blood sample through the counting orifice, and a second syringe coupled in fluid communication with the sensing unit on the inlet side of the counting orifice for simultaneously injecting a sheath of fluid surrounding the sample stream on the inlet side of the counting orifice.

2. An apparatus as defined in claim 1, wherein the pump unit further includes a third syringe coupled in fluid communication with the sensing unit on the outlet side of the counting orifice for aspirating the fluids injected through the counting orifice from the outlet side of the counting orifice.

3. An apparatus as defined in claim 1, wherein the pump unit includes a drive motor coupled to both the first and second syringes for simultaneously actuating the syringes.

4. An apparatus as defined in claim 1, wherein the drive

motor is coupled to a threaded shaft and the threaded shaft is coupled to the first and second syringes, whereupon rotation of the drive motor causes rotation of the threaded shaft and simultaneous actuation of the first and second syringes.

5. An apparatus as defined in claim 4, wherein the drive motor is a stepping motor.

6. An apparatus as defined in claim 4, wherein the pump unit further includes a drive plate coupled between the first and second syringes and the threaded shaft, whereupon rotation of the shaft by the drive motor causes the drive plate to move in the axial direction of the shaft and simultaneously actuate the first and second syringes.

7. An apparatus as defined in claim 1, further including a first-position sensor located approximately at one extreme of permissible movement of the first and second syringes and a second-position sensor located at another approximate extreme of permissible movement of the first and second syringes, each sensor generating a signal when at least one of the first and second syringes reaches the respective extreme position of permissible movement.

8. An apparatus as defined in claim 1, further comprising a valve matrix coupled between the pump unit and the sensing unit

for controlling the flow of fluids between the pump unit and the sensing unit.

9. An apparatus as defined in claim 1, further comprising a control unit coupled to the pump unit and the sensing unit for controlling actuation of the syringes.

10. An apparatus as defined in claim 9, further comprising a first lysing agent chamber and a second lysing agent chamber, each being coupled to the pump unit for aspirating the lysing agents into at least one syringe of the pump unit.

11. An apparatus as defined in claim 10, wherein the control unit includes a database pertaining to predetermined quantities of lysing agents necessary for formulating blood/reagent mixtures for a plurality of species, and is responsive to an input indicating a specific species to control the pump unit to aspirate into at least one of the first and second syringes predetermined quantities of the lysing agents from the first and second lysing chambers corresponding to the respective species.

12. An apparatus as defined in claim 1, further comprising a sample probe coupled to at least one syringe of the pump unit for aspirating a predetermined volume of blood sample into the probe for testing.

13. An apparatus as defined in claim 10, further comprising a mixing chamber coupled in fluid communication with the pump unit for receiving the lysing agents injected by at least one syringe of the pump unit for mixing the lysing agents with a blood sample.

14. An apparatus as defined in claim 1, wherein the sensing unit includes a first injector tube coupled in fluid communication with the first syringe and located on the inlet side of the counting orifice for injecting the sample stream through the counting orifice, and a second injector tube coupled in fluid communication with the second syringe and located on the inlet side of the counting orifice for injecting the sheath fluid adjacent the sample stream.

15. An apparatus as defined in claim 14, wherein the sensing unit defines an inlet chamber coupled in fluid communication with the counting orifice, the first injector tube and the second injector tube, and at least a portion of the second injector tube is oriented substantially on a tangent to a surface defining the inlet chamber for injecting the sheath of fluid in a generally spiral path surrounding the sample stream.

16. An apparatus as defined in claim 2, wherein the sensing unit includes a sheath tube coupled in fluid communication with the third syringe and located on the outlet side of the counting orifice for aspirating a sheath of fluid through the sheath tube

surrounding the sample stream on the outlet side of the counting orifice.

17. An apparatus as defined in claim 16, wherein the sensing unit further defines an exit chamber coupled in fluid communication with the outlet side of the counting orifice for receiving the sample/sheath mixture injected through the counting orifice, and the sheath tube is coupled in fluid communication with the exit chamber, and at least a portion of the sheath tube is oriented substantially on a tangent to a surface defining the exit chamber for directing the sheath of fluid in a generally spiral path surrounding the sample stream on the outlet side of the counting orifice.

18. An apparatus for hematology analysis, comprising:

a sensing unit defining an orifice for receiving a sample stream of blood cells and a sheath of diluent surrounding the sample stream injected into the sensing unit; and

three positive-displacement pumps, each pump including a piston for injecting and aspirating fluid with the respective pump, a first pump coupled in fluid communication with the inlet side of the orifice for injecting the sample stream into the sensing unit on the inlet side of the orifice, a second pump coupled in fluid communication with the inlet side of the orifice for injecting a first sheath of diluent surrounding the sample stream on the inlet side of the orifice, and a third pump coupled in fluid

communication with the sensing unit on the outlet side of the orifice for aspirating a second sheath of diluent through the outlet side of the orifice surrounding the sample stream exiting the orifice.

19. An apparatus as defined in claim 18, further including means for directing at least one of the first and second sheaths of diluent in a substantially spiral path surrounding the sample stream.

20. An apparatus as defined in claim 19, wherein the means for directing includes a first sheath tube coupled on one end in fluid communication with the second pump and coupled on the other end in fluid communication with the inlet side of the orifice, and at least a portion of the first sheath tube is oriented substantially on a tangent to a surface defining a chamber on the inlet side of the orifice.

21. An apparatus as defined in claim 19, wherein the means for directing includes a second sheath tube coupled on one end in fluid communication with the third pump and coupled on the other end in fluid communication with the outlet side of the orifice, and at least a portion of the second sheath tube is oriented substantially on a tangent to a surface defining a chamber on the outlet side of the orifice.

22. An apparatus as defined in claim 18, further comprising a control unit coupled to the three positive-displacement pumps, and including a database pertaining to predetermined quantities of reagents corresponding to blood/reagent mixtures for a plurality of species, the control unit being responsive to an input selecting a particular species to control at least one pump to aspirate the predetermined volumes of reagents corresponding to the respective species.

23. An apparatus as defined in claim 22, further comprising a first chamber containing a first reagent and a second chamber containing a second reagent, the first and second chambers each being coupled in fluid communication with at least one pump for aspirating reagent from each chamber into at least one pump, and a mixing container coupled in fluid communication with at least one pump for injecting the predetermined quantities of reagents from at least one pump into the mixing container.

24. An apparatus for hematology analysis, comprising:

a pump unit comprising at least one positive-displacement pump including a piston for injecting and aspirating fluid with the pump; and

a control unit coupled to the positive-displacement pump, and including a database pertaining to predetermined quantities of reagents corresponding to blood/reagent mixtures for a plurality of species, the control unit being responsive to an input selecting

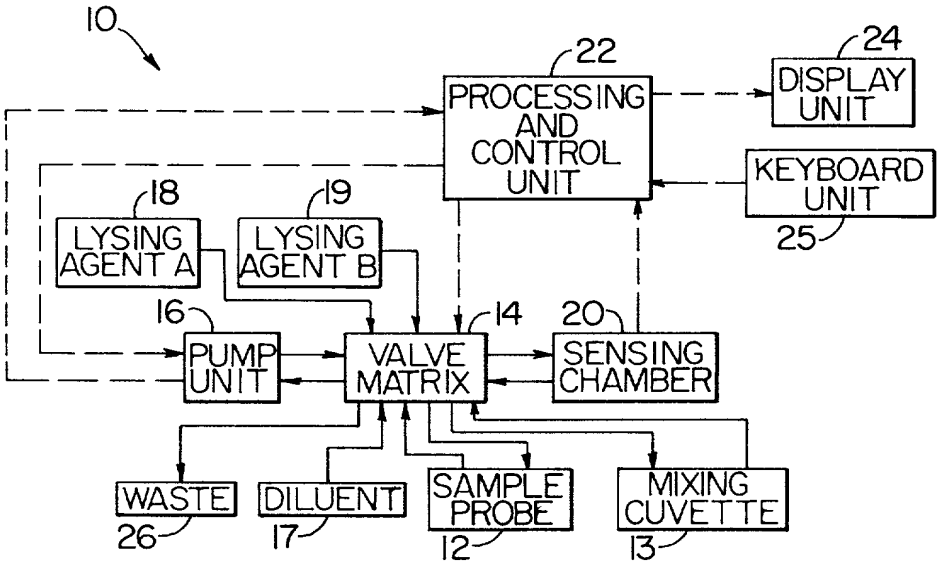
a particular species to control the at least one pump to aspirate the predetermined volumes of reagents corresponding to the respective species into the pump.

25. An apparatus as defined in claim 24, further comprising a first chamber containing a first reagent and a second chamber containing a second reagent, the first and second chambers each being coupled in fluid communication with the at least one pump for aspirating reagent from each chamber into the at least one pump, and a mixing container coupled in fluid communication with the at least one pump for injecting the predetermined quantities of reagents from the at least one pump into the mixing container.

26. An apparatus as defined in claim 24, further comprising a sample probe coupled to the at least one pump, the control unit being responsive to an input selecting a particular species to control the at least one pump to aspirate a predetermined volume of whole blood sample corresponding to the respective species into the pump.

Abstract of the Disclosure

An apparatus is provided for hematology testing, which has a sensing unit defining a counting orifice for the flow of a blood sample through the counting orifice to analyze the blood sample, and a pump unit having three syringes. A first syringe is coupled in fluid communication with the sensing unit on the inlet side of the counting orifice for injecting a stream of blood sample through the counting orifice. A second syringe is coupled in fluid communication with the sensing chamber on the inlet side of the counting orifice for simultaneously injecting a sheath of fluid surrounding the sample stream on the inlet side of the counting orifice. And a third syringe is coupled to the sensing chamber on the outlet side of the counting orifice for aspirating a sheath of fluid from the sensing chamber surrounding the sample stream on the outlet side of the counting orifice.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DISPLAY UNIT
KEYBOARD UNIT
25
18
LYSING AGENT A
19
LYSING AGENT B
16
PUMP UNIT
14
VALVE MATRIX
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SENSING CHAMBER
WASTE
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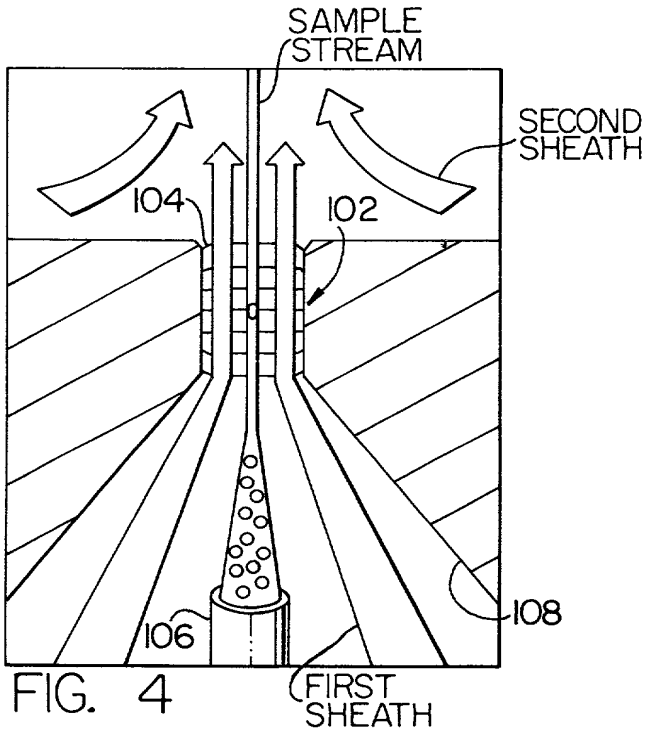
DILUENT
17
SAMPLE PROBE
12
MIXING CUVETTE
13

FIG. 1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FIRST SHEATH

FIG. 4

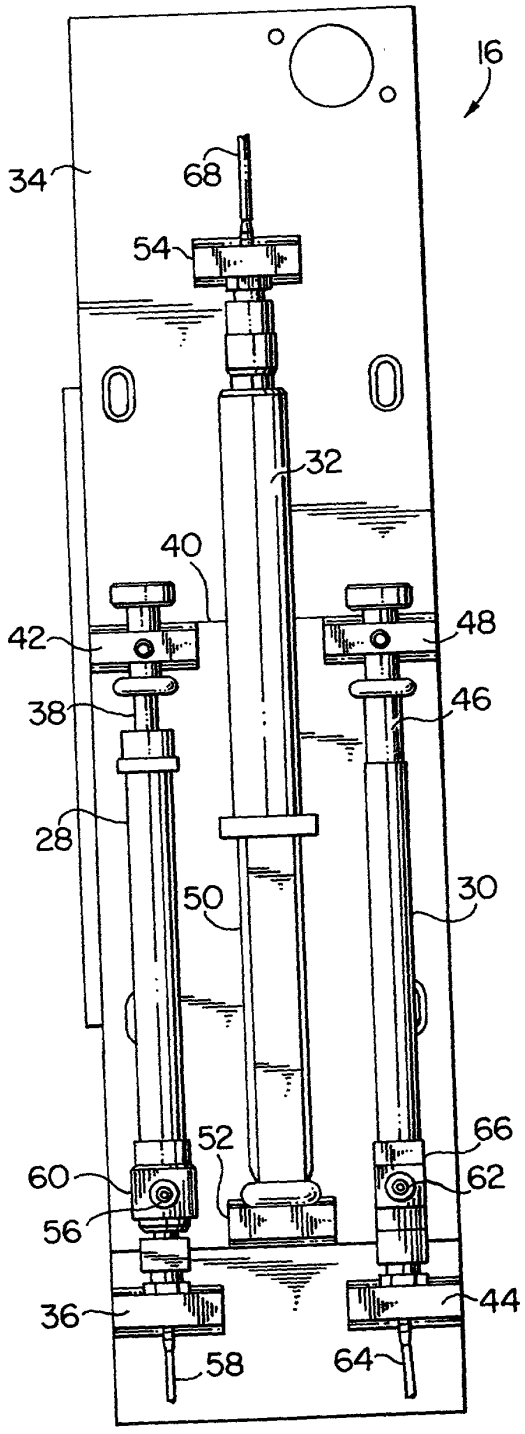

FIG. 2

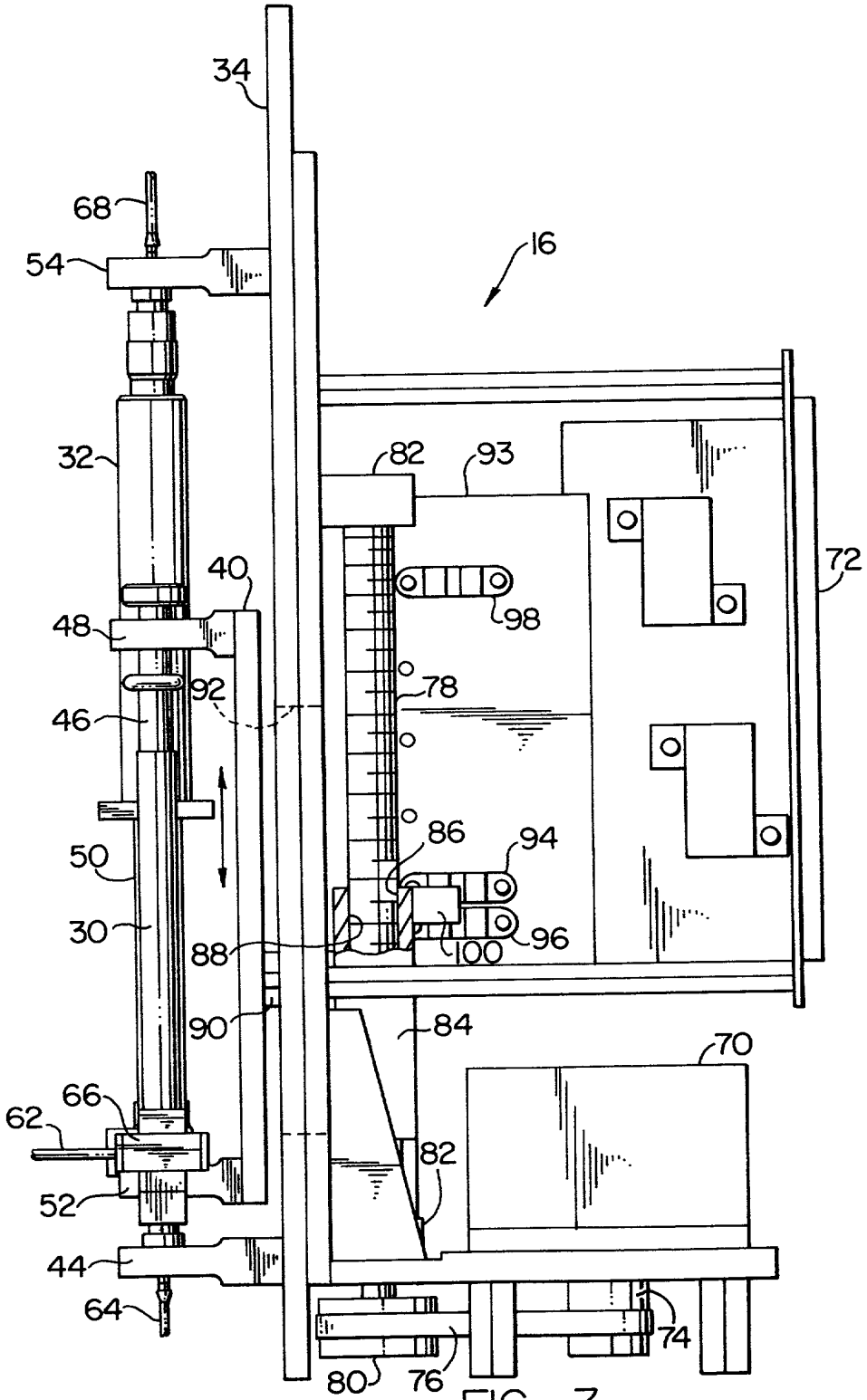
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FIG. 3

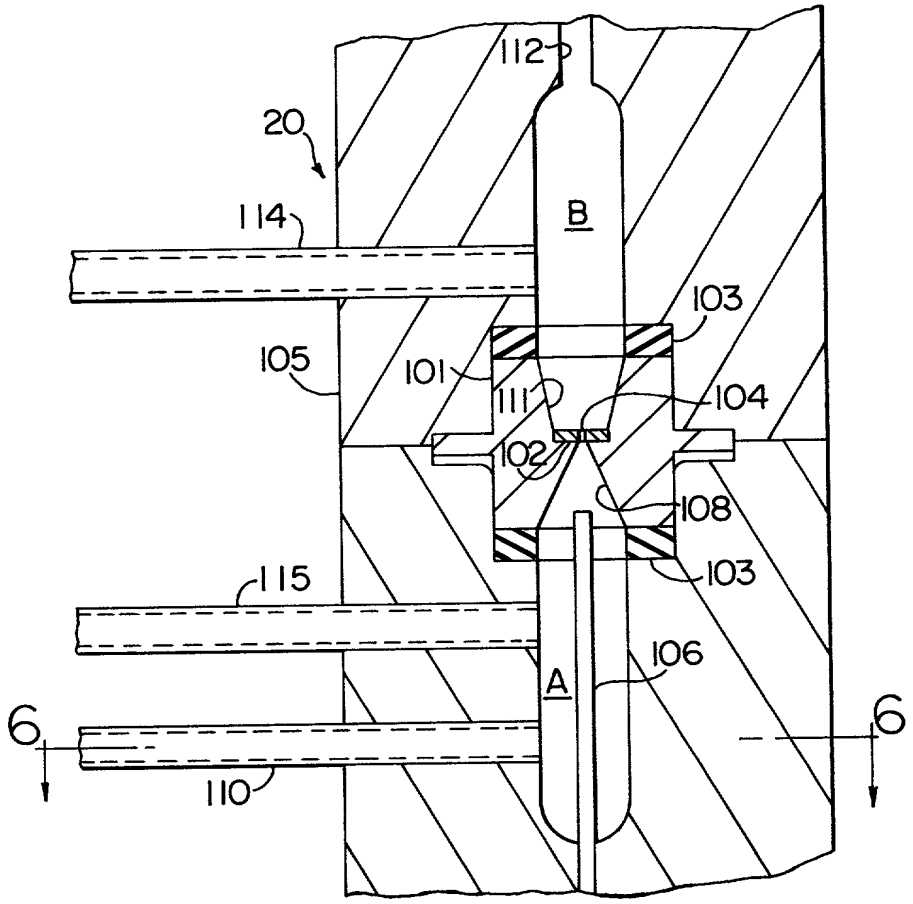
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FIG. 5

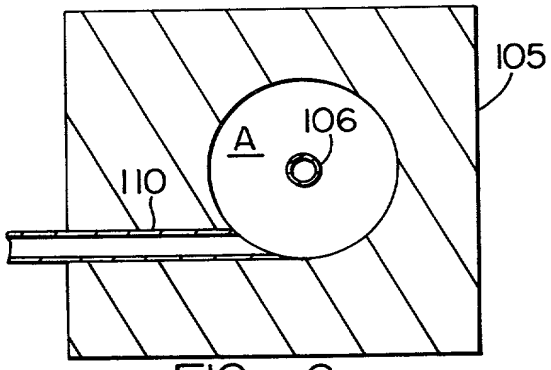
105
A
106
110

FIG. 6

ATTORNEY DOCKET NO. CDC-104

Declaration, Power of Attorney, and Petition

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled APPARATUS FOR PUMPING AND DIRECTING FLUIDS FOR HEMATOLOGY TESTING

_____ the specification of which

(check one) ☒ is attached hereto. ☐ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above, and that it contains a full, clear, concise and exact description of the subject matter for which a patent is sought.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

(Number)	(Country)	Day/month/year filed	Priority claimed	
			☐ Yes	☐ No
			☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing date)	(Status) (patented, pending, abandoned)

☐ (Check if applicable) I hereby authorize the U.S. attorneys or agents named herein to accept and follow instructions from _____ as to any action to be taken in the Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys or agents named herein and myself. In the event of a change, I will notify in writing the U.S. attorney or agent named herein.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint:

Barry Kramer, Reg. No. 20,622
Allen D. Brufsky, Reg. No. 21,056
~~Julia D. Harby, Reg. No. 33,192~~
Mark D. Giarratana, Reg. No. 32,615

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my attorney with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Wherefore I pray that Letters Patent be granted to me for the invention or discovery described and claimed in the foregoing specification and claims, and I hereby subscribe my name to the foregoing specification and claims, declaration, power of attorney, and this petition.

Full name of sole or first inventor: Edward Lawrence Carver, Jr.
Inventor's signature: Edward L Carver Jr Date: 1-20-93
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Citizenship: United States of America
Post Office Address: 18 Lisa Drive, Oxford, Connecticut 06478

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Second Inventor's signature: David Charles DeCava Date: 1-20-93
Residence: 35 Sioux Drive, Oxford, Connecticut 06478
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Full name of fourth joint inventor, if any
Fourth Inventor's signature Date
Residence
Citizenship
Post Office Address

Full name of fifth joint inventor, if any
Fifth Inventor's signature Date
Residence
Citizenship
Post Office Address

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of	)	
	)	
Edward L. Carver, Jr., et al	)	
	)	Examiner: R. Torres
on APPARATUS FOR PUMPING AND DIRECTING FLUID FOR HEMATOLOGY TESTING	)	Group Art Unit: 1809
	)	
Serial No.: 08/007,111	)	
	)	
Filed On: January 21, 1993	)	(Our Docket No. 4537-01)

Hartford, Connecticut,

Hon. Assistant Secretary and Commissioner
of Patents and Trademarks
Washington, D. C. 20231

REVOCATION OF PRIOR POWER OF ATTORNEY AND APPOINTMENT OF NEW POWER OF ATTORNEY BY ASSIGNEE OF ENTIRE INTEREST

Madam/Sir:

As assignee of record of the entire interest of the above-identified patent application, all powers of attorney previously given are hereby revoked, and the following attorneys are hereby appointed to prosecute and transact all business in the United States Patent and Trademark Office connected therewith:

Theodore R. Paulding, Registration No. 19,294; Donald K. Huber, Registration No. 18,686; John C. Hilton, Registration No. 22,965; Frederick J. Haesche, Registration No. 24,529; John C. Linderman, Registration No. 24,420; Jack M. Pasquale, Registration No. 31,052; J. Kevin Grogan, Registration No.